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SUBCHRONIC ORAL TOXICITY OF THE INSECT REPELLENT N,N-DIETHYL-M---ETC(U)
FEB 80 E A HAIGHT, J G HARVEY, A W SINGER

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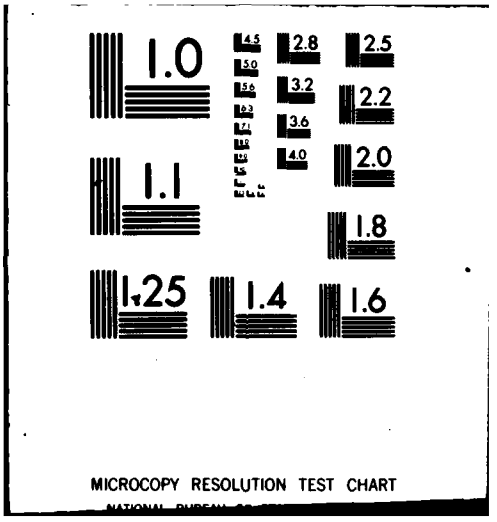
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LEVEL II

# UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

## ABERDEEN PROVING GROUND, MD 21010

PHASE 5
SUBCHRONIC ORAL TOXICITY STUDY OF THE
INSECT REPELLENT N,N-DIETHYL-M-TOLUAMIDE (M-DET)
75-51-0034-80
SEPTEMBER 1978 - MAY 1979



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## REPORT DOCUMENTATION PAGE

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| | |
|---|---|
| 1. REPORT NUMBER: 75-51-0034-80 | 2. GOVT ACCESSION NO. |
| 3. RECIPIENT'S CATALOG NUMBER | |
| 4. TITLE (and Subtitle): Subchronic Oral Toxicity of the Insect Repellent N,N-Diethyl-m-Toluamide (m-DET), Study No. 75-51-0034-80, September 1978 - May 1979 | 5. TYPE OF REPORT & PERIOD COVERED: Special Study ~~Sep 78 - May 79~~ |
| | 6. PERFORMING ORG. REPORT NUMBER |
| 7. AUTHOR(s): EVERETT A. HAIGHT, JOHN G. HARVEY, JR., ALLEN W. SINGER, CONRAD R. POPE, LTC, VC | 8. CONTRACT OR GRANT NUMBER(s) |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS: US Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD 21010 | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS |
| 11. CONTROLLING OFFICE NAME AND ADDRESS: Commander, US Army Health Services Command, Fort Sam Houston, TX 78234 | 12. REPORT DATE: Sep 78 - May 79<br>13. NUMBER OF PAGES: 23 |
| 14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) | 15. SECURITY CLASS. (of this report): Unclassified<br>15a. DECLASSIFICATION/DOWNGRADING SCHEDULE |

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

| | |
|---|---|
| N,N-Diethyl-m-Toluamide | Serum |
| m-DET | Cholesterol |
| Pest Repellent | Triglyceride |
| Subchronic Oral | Calcium |
| Male Rabbits | |

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A subchronic oral toxicity evaluation of the technical grade compound N,N-diethyl-m-toluamide (m-DET) was conducted by this Agency using rabbits. The high dose group 528 mg/kg (1/3 $LD_{50}$) showed a progressive decrease in body weight throughout the study. Serum calcium levels were decreased, and cholesterol and triglyceride levels were increased significantly for this same group. No other toxic signs were observed during the 15-day treatment period.

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Weeks/lm/AUTOVON
584-3980

HSE-LT/WP

6 FEB 1980

SUBJECT: Phase 5, Subchronic Oral Toxicity of the Insect Repellent N,N-Diethyl-m-Toluamide (m-DET) Study No. 75-51-0034-80, September 1978 - May 1979

Executive Secretary
Armed Forces Pest Control Board
Forest Glen Section, WRAMC
Washington, DC 20012

A summary of the pertinent findings and recommendations of the inclosed report follows:

a. A subchronic oral toxicity evaluation of the technical grade compound N,N-diethyl-m-toluamide (m-DET) was conducted by this Agency using rabbits. The high dose group 528 mg/kg (1/3 $LD_{50}$) showed a progressive decrease in body weight throughout the study. Serum calcium levels were decreased, and cholesterol and triglyceride levels were increased significantly for this same group. No other toxic signs were observed during the 15-day treatment period.

b. It was recommended that the weight loss and serum chemistry changes be addressed during future studies undertaken during the continuing process of registration of this pest repellent with the Environmental Protection Agency.

FOR THE COMMANDER:

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as

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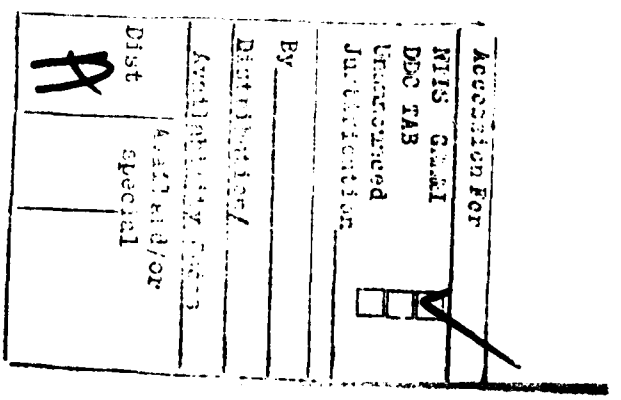

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP

# SUBCHRONIC ORAL TOXICITY STUDY OF THE INSECT REPELLENT N,N-DIETHYL-M-TOLUAMIDE (M-DET)†*

75-51-0034-80
SEPTEMBER 1978 - MAY 1979

## 1. AUTHORITY.

a. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the US Department of Agriculture, Agricultural Research, Science and Education Administration; titled, Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

b. Letter, Armed Forces Pest Control Board, 17 March 1977, subject: Request for Toxicological Evaluation.

## 2. REFERENCES.

a. Toxicology Division Procedural Guide, USAEHA, 1972 revised 1976.

b. O-I-503E, 28 March 1975, Proposed Federal Specification Insect Repellent, Clothing and Personal Application.

3. PURPOSE. A literature search revealed no data on major enzyme systems which might be affected by chronic application of N,N-diethyl-m-toluamide in animals. This study was conducted to determine which, if any, organs or enzymes systems would be adversely affected by oral administration of m-DET in rabbits. Information gained by this study could then be used to further clarify future subchronic studies required by the Environmental Protection Agency in registration of this pest repellent.

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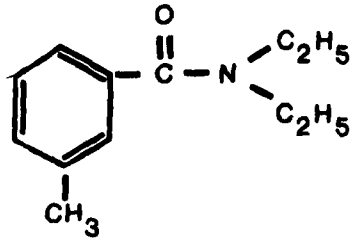
†* The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education and Welfare, Publication No. (NIH) 78-23, revised 1978.
* This study was performed in animal facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Study No. 75-51-0034-80, Sep 78 - May 79

4. BACKGROUND.

a. N,N-diethyl-m-toluamide is a standard US Army insect repellent, with its formulation described in Fed Spec O-I-503E. Henceforth, N,N-diethyl-m-toluamide will be referred to as either diethyltoluamide or m-DET. The toxicity of this compared was previously reported by Ambroset in 1959. Unfortunately, the original laboratory data are no longer available for regulatory review in support of registration of m-DET as an insect repellent. This deficiency necessitated the development of additional laboratory data on the health effects of technical grade m-DET in animals. The study of the toxicity of orally administered repellent to rabbits was started 1 September 1978 and completed 21 September 1978. The histopathologic examination of tissues and organs and data handling was completed 1 April 1979. Our sample of the technical grade compound was manufactured by Hardwicke Chemical Company, Elgin, SC 29045, and packaged by McLaughlin Gormley King Company, 8810 Tenth Avenue, North Minneapolis, MN 55427. It was labeled as lot number 7141, with the active ingredients being approximately 95 percent as the meta isomer and 5 percent other isomers. M-DET has the following chemical structure:



b. Recent studies in our laboratory have shown that the technical grade m-DET produced only a mild primary irritation when applied to the intact and abraded skin of rabbits. The oral $LD_{50}$ in female rats for the technical grade material was 2420 mg/kg (95 percent C.L.* 1940-3020 mg/kg) and in male rats 3290 mg/kg (95 percent C.L. 2660-4066 mg/kg). The oral $LD_{50}$ in female rats for m-DET administered in corn oil was 2170 mg/kg (95 percent C.L. 1720-2730 mg/kg), while in male rats it was 3160 mg/kg (95 percent C.L. 2640-3770 mg/kg). These $LD_{50}$ values show an apparent difference in response levels between the sexes, with females somewhat more sensitive than male rats. No change in toxicity appears to be due to the presence of the unsaturated corn oil when given with the technical material.

5. PROCEDURE. A subchronic oral toxicity evaluation of technical grade m-DET was conducted using male, New Zealand White rabbits.† The acute oral

---

† Anthony M. Ambrose, Pharmacological and Toxicologic Studies on N,N-Diethyl-m-toluamide (m-Det), Toxicol. Appl. Pharmacol., L. 97-115 (1959)
* Confidence limits
† Purchased from Dutchland Laboratory Animals Inc., Denver, PA.

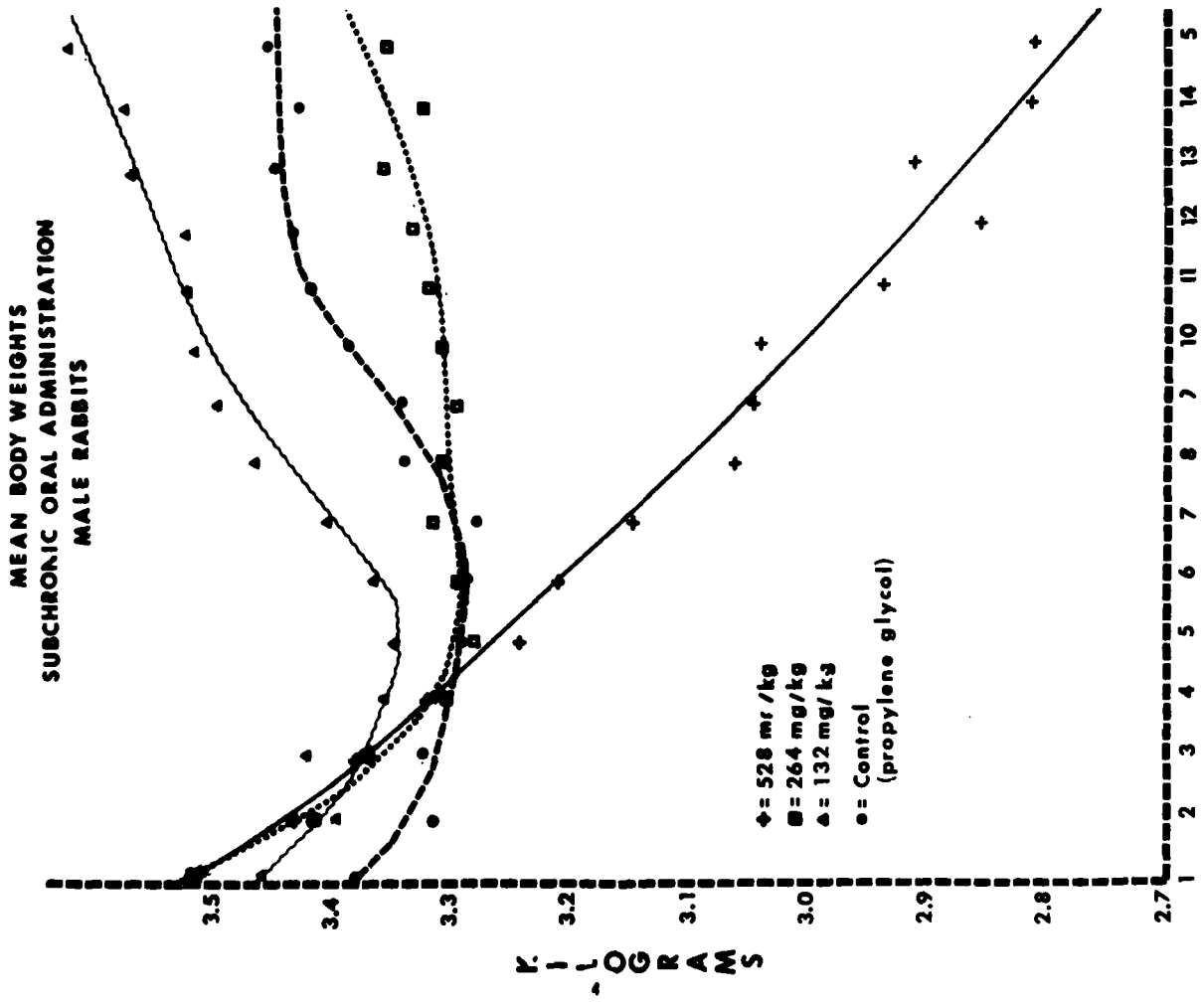
$LD_{50}$ in New Zealand White rabbits of technical grade m-DET had previously been determined to be 1585 mg/kg (95 percent C.L. 1205-2084 mg/kg). In the subchronic study, three groups of six rabbits each received daily oral m-DET dosages for 15 days of 1/3, 1/6, and 1/12 fractions of the acute oral $LD_{50}$. A control group of six rabbits received daily a 0.5 ml/kg dosage of propylene glycol. All animals were weighed daily and observed twice daily for signs. Blood was taken from the central ear artery at 24 hours following the first dosing and after 7 and 14 days of dosing for clinical chemistry determination. An Abbott Bichromatic Analyzer 100 (ABA-100) was used for clinical chemistry determinations of serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), lactate dehydrogenase (LDH), alkaline phosphatase, gamma glutamyl transpeptidase (GGTP), glucose, blood urea nitrogen (BUN), total bilirubin, total protein, sodium, potassium, calcium, cholesterol, and triglycerides. At necropsy, following 15 days of daily adminstration of m-DET, a comparison was made of the relative organ weights of lung, liver, kidney and testes from the various dosing groups. In addition, the following organs and tissues were taken for histopathologic examination: eye, brain, lung, testes, kidney, liver, spleen, heart, stomach, pancreas, large and small intestine, skeletal muscle, bladder, bone, skin, trachea, esophagus, cecum, adrenals, thymus, and bone marrow.

6. RESULTS.

a. Rabbits receiving 528 mg/kg (1/3 $LD_{50}$) m-DET showed a progressive decrease in body weight, becoming significantly different from the other three groups at day seven.

b. Rabbits receiving propylene glycol, 264 mg/kg (1/6 $LD_{50}$) and 132 mg/kg (1/12 $LD_{50}$) showed a slight decrease in body weight throughout the first few days but apparently accommodated to the insult and/or handling after 6 days. Thereafter, the body weights of rabbits in these groups progressively increased. These changes are presented graphically in the Figure.

c. The mean organ-to-body weight ratios from the various groups are presented in Table 1. The relative kidney weights increased in the group receiving the highest dosage (528 mg/kg), and the lung weights increased over controls in animals receiving the middle dosage (264 mg/kg). These changes may be artifacts and have no real meaning since no collaborating changes or lesions could be found upon microscopic examination of the respective tissues.


MEAN BODY WEIGHTS
SUBCHRONIC ORAL ADMINISTRATION
MALE RABBITS
KILOGRAMS
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS
◆= 528 mg/kg
■= 264 mg/kg
▲= 132 mg/kg
●= Control
(propylene glycol)

FIGURE

d. Histopathologically, rare to minimal fatty changes were observed in hepatocytes in a suggestive dose related pattern. In addition, the bile ducts in the livers from all groups were particularly prominent. This latter finding is a common incidental finding in conventional source rabbits, and is due to previous infection of Eimeria sp. Interstitial nephritis was present to a minor degree in all animals. A few lung sections in all groups had areas of pneumonitis. None of the cited lesions appeared to be masking compound related lesions. Details of the histopathology are presented in Table 2.

e. Results from serum clinical chemistries are presented in Tables 3-16. The test compound, m-DET, produced a significant decrease in calcium and an increase in cholesterol and triglyceride levels at the highest dosage (528 mg/kg). The cholesterol increase and calcium decrease occurred after 7 days of daily oral dosing and continued throughout the test. The triglycerides were significantly increased after day 1 and day 14 of exposure. These changes in clinical chemistry parameters are not targeted to specific organ functions and may be reflections of the decreases in body weight of the rabbits at the highest dosage.

7. DISCUSSION. Data from these studies indicate that a relatively short oral administration of m-DET can produce a definite change in body weight at the dosage level of 528 mg/kg/day. This change in body weight probably influences the changes seen in the clinical chemistry parameters of cholesterol, calcium and triglyceride levels. Although no target organ system or function seems to be affected, these data do give background changes that should be investigated in future subchronic studies.

8. CONCLUSION. Daily high oral intake of technical grade m-DET (528 mg/kg/day) causes, in 15 days, decreases in body weight and serum calcium and increases in serum cholesterol and triglyceride levels.

9. **RECOMMENDATION.** Additional subchronic studies will be developed in the continuing effort to register this pest repellent with the Environmental Protection Agency. The observed decreases in body weight and serum calcium, and increases in serum cholesterol and triglycerides should be further evaluated as part of this effort.

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Study No. 75-51-0034-80, Sep 78 - May 79

APPENDIX

Table 1 - Mean Organ-to-Body Weight Ratios

Table 2 - Subacute Oral

Table 3 - 6 - Results from Serum Clinical Chemistries

Study No. 75-51-0034-80, Sep 78 - May 79

TABLE 1. SUMMARY OF ORGAN-TO-BODY WEIGHT RATIOS (NEW ZEALAND WHITE RABBITS)

| Dosage | Terminal Body Weight (gm) + SD | Mean Organ-to-Body Weight Ratios (gm/100 gms body weight) + SD | | | |
|---|---|---|---|---|---|
| M-Det | | Lung | Liver | Kidney | Testes |
| 528 mg/kg | 2750* ±220 | 0.65 ±0.26 | 2.86 ±0.73 | 0.78* ±0.06 | 0.22 ±0.07 |
| 264 mg/kg | 3350 ±380 | 0.81* ±0.18 | 3.26 ±0.50 | 0.58 ±0.05 | 0.19 ±0.04 |
| 132 mg/kg | 3590 ±530 | 0.58 ±0.30 | 3.25 ±0.36 | 0.62 ±0.08 | 0.18 ±0.04 |
| Control (Propylene glycol) 0.5 ml/kg | 3450 ±270 | 0.43 ±0.04 | 3.04 ±0.19 | 0.56 ±0.04 | 0.15 ±0.04 |

* Sig @ P>.05

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TABLE 2. SUBACUTE ORAL (PB-798-78 to PB-816-78)

| PB- -78 | Rabbit No. | Eye | Brain | Heart[x] | Trachea[x] | Lung | Esophagus[x] | Stomach | Small Intestine | Pancreas | Cecum | Large Intestine | Liver | Kidney | Spleen | Adrenal | Bladder | Skeletal Muscle | Bone | Bone Marrow | Skin | Thymus | Testes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | | | | | | | | | | | | | | | | | | | | | | | |
| 802 | 128 | ✓ | ✓ | ✓ | ✓ | 1a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | X | ✓ | ✓ |
| 801 | 122 | ✓ | ✓ | ✓ | ✓ | 1b | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | X | ✓ | ✓ |
| 800 | 113 | ✓ | ✓ | ✓ | ✓ | 1a 4 | ✓ | ✓ | ✓ | ✓ | 6 | ✓ | 7 | 5 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 799 | 109 | ✓ | ✓ | ✓ | 2 | 1b | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 3a |
| 798 | 101 | ✓ | X | X | X | 1b | X | X | X | X | X | X | X | X | X | X | ✓ | ✓ | X | X | X | ✓ | ✓ |
| **528 mg/kg M-Det** | | | | | | | | | | | | | | | | | | | | | | | |
| 803 | 106 | ✓ | ✓ | ✓ | ✓ | 1a 4 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 8 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 3b |
| 804 | 110 | ✓ | ✓ | ✓ | ✓ | 1a 4a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 8a | 5a 9 10 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 805 | 129 | ✓ | ✓ | ✓ | ✓ | 1a 4a 11 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | * | 5 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **264 mg/kg M-Det** | | | | | | | | | | | | | | | | | | | | | | | |
| 806 | 103 | ✓ | ✓ | ✓ | 2a | 1a 4a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 807 | 107 | ✓ | ✓ | ✓ | ✓ | 2a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 808 | 111 | ✓ | ✓ | ✓ | ✓ | 1a 4a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 809 | 115 | ✓ | ✓ | ✓ | ✓ | 1a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 5a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 810 | 126 | ✓ | ✓ | ✓ | ✓ | 1 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 811 | 131 | ✓ | ✓ | ✓ | ✓ | 1a 11 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 8 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **132 mg/kg M-Det** | | | | | | | | | | | | | | | | | | | | | | | |
| 812 | 104 | ✓ | ✓ | ✓ | ✓ | 1a 4a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 813 | 108 | ✓ | ✓ | ✓ | ✓ | 1a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 814 | 116 | ✓ | ✓ | ✓ | ✓ | 2b 4b | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 815 | 119 | ✓ | ✓ | ✓ | ✓ | 1a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 816 | 127 | ✓ | 12 | ✓ | ✓ | 1a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 5a | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |

See footnotes on page 9.

COMMENT: Compound related lesions in a suggestive dose response pattern were observed in the liver. No other organs gave evidence of compound related lesions. The liver findings consist of rare to minimal fatty change observed in hepatocytes. This change is primarily midzonal, but clear vacuolated hepatocytes can also be seen in central and portal areas. This change was observed in 0/4 controls, 2/3 528 mg/kg, 1/6 264 mg/kg and 0/5 132 mg/kg. The bile ducts in the liver from all four groups of rats were prominent. Interstitial nephritis was present to a minor degree in all four groups. A few sections of lung in all groups had areas of pneumonitis.

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FOOTNOTES FOR TABLE 2

SUBACUTE ORAL

PB-798-78 to PB-816-78

X - No tissue

✓ - Normal

1 - Lymphoid nodules, rare, lung

1a - Lymphoid nodules, minimal, lung

1b - Lymphoid nodules, moderate, lung

2 - Lymphoid infiltration, rare, trachea

2a - Lymphoid infiltration, minimal, trachea

2b - Lymphoid infiltration, moderate, trachea

3a - atrophy with spermatidic giant cells, diffuse seminiferous tubules, testes, minimal

3b - atrophy with spermatidic giant cells, diffuse seminiferous tubules, testes, moderate

4 - Pneumonitis, rare, lung

4a - Pneumonitis, minimal, lung

4b - Pneumonitis, moderate, lung

5 - Interstitial nephritis, minimal, kidney

5a - Interstitial nephritis, moderate, kidney

6 - Nematodiasis, rare, cecum

7 - Bile duct hyperplasia with associated lymphocytosis, minimal, liver

8 - Fatty change, diffuse, liver, rare

8a - Fatty change, diffuse, liver, minimal

9 - Tubular nephrosis, minimal, kidney

10 - Proteinaceous casts, minimal-moderate, tubules, kidney

11 - Interstitial lobar pneumonia, diffuse, moderate, lung

12 - Meningoencephalitis, minimal, cerebrum, midbrain

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TABLE 3. SUMMARY OF SERUM SGOT VALUES (INTERNATIONAL UNITS/LITER)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 21.0 ±7.7 | 27.9 ±16.4 | 24.0 ±6.1 | 22.0 ±14.7 |
| Oral Dose 264 mg/kg | 14.5 ±4.3 | 18.3 ±8.9 | 21.2 ±4.6 | 14.0 ±3.2 |
| Oral Dose 132 mg/kg | 24.0 ±18.5 | 23.2 ±17.7 | 21.1 ±12.2 | 18.2 ±16.3 |
| Control Propylene glycol 0.5 ml/kg | 20.8 ±13.8 | 23.7 ±11.3 | 29.2 ±17.9 | 25.6 ±16.0 |

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TABLE 4. SUMMARY OF SERUM SGPT VALUES (INTERNATIONAL UNITS/LITER)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 43.5 ±17.6 | 52.0 ±17.1 | 35.6 ±12.5 | 39.8 ±17.7 |
| Oral Dose 264 mg/kg | 40.7 ±6.4 | 54.2 ±8.9 | 40.5 ±14.7 | 38.8 ±10.7 |
| Oral Dose 132 mg/kg | 50.7 ±22.4 | 56.2 ±22.1 | 45.8 ±20.9 | 47.2 ±20.8 |
| Control Propylene glycol 0.5 ml/kg | 42.0 ±30.5 | 57.5 ±34.7 | 58.6 ±51.6 | 51.8 ±41.5 |

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TABLE 5. SUMMARY OF SERUM TOTAL LDH VALUES (INTERNATIONAL UNITS/LITER)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 144.2 ±64.6 | 151.5 ±62.1 | 218.4 ±76.5 | 160.3 ±40.8 |
| Oral Dose 264 mg/kg | 123.3 ±57.0 | 92.3 ±27.6 | 159.3 ±48.1 | 94.2 ±27.4 |
| Oral Dose 132 mg/kg | 130.5 ±54.3 | 85.5 ±18.5 | 101.2 ±31.9 | 88.8 ±28.8 |
| Control Propylene glycol 0.5 ml/kg | 172.5 ±111.6 | 165.8 ±80.4 | 166.6 ±57.8 | 134.0 ±37.6 |

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TABLE 6. SUMMARY OF SERUM ALKALINE PHOSPHATASE VALUES (INTERNATIONAL UNITS/LITER)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 132.0 ±42.7 | 107.7 ±36.3 | 72.6 ±25.8 | 56.8 ±28.6 |
| Oral Dose 264 mg/kg | 106.3 ±34.1 | 102.0 ±32.6 | 74.8 ±27.2 | 74.2 ±15.3 |
| Oral Dose 132 mg/kg | 138.0 ±40.6 | 114.7 ±30.1 | 96.8 ±20.3 | 102.4 ±21.1 |
| Control Propylene glycol 0.5 ml/kg | 103.5 ±12.9 | 99.3 ±17.6 | 105.4 ±18.8 | 84.8 ±4.1 |

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TABLE 7. SUMMARY OF SERUM GGTP* VALUES (INTERNATIONAL UNITS/LITER)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 5.3 ±0.8 | 4.7 ±1.5 | 3.6 ±1.7 | 3.8 ±1.0 |
| Oral Dose 264 mg/kg | 5.0 ±1.4 | 5.2 ±1.5 | 2.5 ±1.9 | 2.2 ±1.6 |
| Oral Dose 132 mg/kg | 5.7 ±1.9 | 5.7 ±2.2 | 3.2 ±0.8 | 3.8 ±1.3 |
| Control Propylene glycol 0.5 ml/kg | 4.3 ±1.5 | 4.7 ±1.5 | 1.8 ±0.8 | 2.6 ±2.1 |

* Gamma Glutamyl Transpeptidase

Study No. 75-51-0034-80, Sep 78 - May 79

TABLE 8. SUMMARY OF SERUM TOTAL BILIRUBIN VALUES (INTERNATIONAL UNITS/LITER)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 0.3 ±0.0 | 0.3 ±0.1 | 0.4 ±0.1 | 0.9 ±0.7 |
| Oral Dose 264 mg/kg | 0.3 ±0.0 | 0.3 ±0.1 | 0.3 ±0.1 | 0.3 ±0.1 |
| Oral Dose 132 mg/kg | 0.3 ±0.0 | 0.3 ±0.0 | 0.4 ±0.1 | 0.3 ±0.1 |
| Control Propylene glycol 0.5 ml/kg | 0.3 ±0.0 | 0.3 ±0.0 | 0.3 ±0.1 | 0.3 ±0.1 |

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TABLE 9. SUMMARY OF SERUM CALCIUM (mg/dl)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 15.08 ±0.77 | 14.80 ±0.56 | *12.99 ±0.80 | *13.03 ±1.73 |
| Oral Dose 264 mg/kg | 15.03 ±0.51 | 14.07 ±0.86 | 13.90 ±0.73 | 14.53 ±0.70 |
| Oral Dose 132 mg/kg | 15.41 ±0.68 | 15.05 ±0.61 | 14.14 ±0.37 | 15.00 ±0.51 |
| Control Propylene glycol 0.5 ml/kg | 14.91 ±0.49 | 15.01 ±0.49 | 14.39 ±0.51 | 15.10 ±0.48 |

* Sig @ P>0.02

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TABLE 10. SUMMARY OF SERUM TOTAL PROTEIN VALUES (g/dl)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 6.35 ±0.27 | 6.42 ±0.47 | 6.57 ±0.82 | 6.47 ±0.70 |
| Oral Dose 264 mg/kg | 6.05 ±0.46 | 6.32 ±0.57 | 6.37 ±0.89 | 6.41 ±0.44 |
| Oral Dose 132 mg/kg | 6.41 ±0.45 | 6.39 ±0.17 | 6.34 ±0.77 | 6.83 ±0.47 |
| Control Propylene glycol 0.5 ml/kg | 6.22 ±0.44 | 6.37 ±0.63 | 6.61 ±0.60 | 6.37 ±0.59 |

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TABLE 11. SUMMARY OF SERUM CHOLESTEROL (mg/dl)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 41.17 ±6.97 | 45.83 ±10.13 | *92.33 ±21.80 | *190.50 89.85 |
| Oral Dose 264 mg/kg | 52.33 ±18.34 | 47.83 ±14.95 | 60.50 ±18.10 | 53.50 ±19.49 |
| Oral Dose 132 mg/kg | 41.50 ±6.98 | 38.17 ±5.95 | 53.20 ±12.62 | 38.40 ±7.83 |
| Control Propylene glycol 0.5 ml/kg | 49.67 ±18.35 | 48.83 ±18.55 | 52.40 ±16.68 | 39.20 ±15.90 |

* Sig @ P>0.02

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TABLE 12. SUMMARY OF SERUM GLUCOSE VALUES (mg/dl)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 154.83 ±29.98 | 149.00 ±25.47 | 146.00 ±32.89 | 169.00 ±24.58 |
| Oral Dose 264 mg/kg | 144.00 ±16.49 | 139.00 ±10.16 | 153.67 ±10.41 | 166.83 ±15.17 |
| Oral Dose 132 mg/kg | 153.83 ±39.17 | 148.00 ±18.49 | 146.20 ±7.60 | 158.00 ±12.14 |
| Control Propylene glycol 0.5 ml/kg | 139.17 ±7.31 | 123.00 ±6.90 | 130.20 ±8.38 | 138.20 ±11.52 |

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TABLE 13. SUMMARY OF SERUM BUN VALUES (mg/dl)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 16.22 ±2.98 | 14.87 ±2.38 | 19.44 ±10.03 | 32.10 18.77 |
| Oral Dose 264 mg/kg | 16.97 ±2.54 | 15.57 ±2.19 | 14.80 ±4.49 | 15.38 ±2.22 |
| Oral Dose 132 mg/kg | 16.30 ±1.62 | 12.02 ±1.69 | 12.14 ±1.32 | 17.00 ±1.31 |
| Control Propylene gylcol 0.5 ml/kg | 15.95 ±2.07 | 14.15 ±1.94 | 14.34 ±2.09 | 16.30 ±2.16 |

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TABLE 14. SUMMARY SERUM TRIGLYCERIDE VALUES (mg/dl)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 143.17 ±48.89 | *254.33 ±120.41 | †319.20 ±212.24 | *510.50 ±187.40 |
| Oral Dose 264 mg/kg | 118.17 ±77.13 | 101.17 ±26.35 | 213.50 ±95.85 | 200.67 ±142.21 |
| Oral Dose 132 mg/kg | 147.83 ±86.72 | 149.83 ±90.70 | 203.2 ±197.13 | 116.00 ±39.35 |
| Control Propylene glycol 0.5 ml/kg | 131.17 ±67.19 | 131.17 ±38.24 | 173.4 ±65.39 | 172.2 ±79.46 |

* Sig @ P>0.02

† Not significant but clinically elevated.

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TABLE 15. SUMMARY OF SERUM SODIUM VALUES (mEq/l)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 145.50 ±2.51 | 146.83 ±1.72 | 143.40 ±4.51 | 132.75 ±12.15 |
| Oral Dose 264 mg/kg | 145.50 ±3.99 | 146.50 ±2.74 | 146.67 ±3.44 | 146.67 ±1.63 |
| Oral Dose 132 mg/kg | 145.33 ±1.63 | 146.83 ±2.93 | 145.80 ±1.10 | 147.20 ±1.10 |
| Control Propylene glycol 0.5 ml/kg | 146.50 ±3.15 | 147.00 ±2.83 | 147.00 ±2.55 | 147.40 ±1.52 |

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TABLE 16. SUMMARY OF SERUM POTASSIUM VALUES (mEq/l)

| | Mean of 7 Pretest Values + SD | Period of Test Mean + SD | | |
|---|---|---|---|---|
| Type Application | | Day 1 | Day 7 | Day 14 |
| Oral Dose 528 mg/kg | 5.48 ±0.78 | 5.08 ±0.42 | 4.06 ±0.87 | 3.40 ±1.26 |
| Oral Dose 264 mg/kg | 5.65 ±0.96 | 4.75 ±0.43 | 4.82 ±0.83 | 5.27 ±0.40 |
| Oral Dose 132 mg/kg | 5.65 ±0.93 | 5.35 ±0.83 | 5.02 ±0.19 | 5.50 ±0.42 |
| Control Propylene gylcol 0.5 ml/kg | 5.22 ±0.38 | 4.98 ±0.52 | 4.68 ±0.34 | 5.06 ±0.27 |